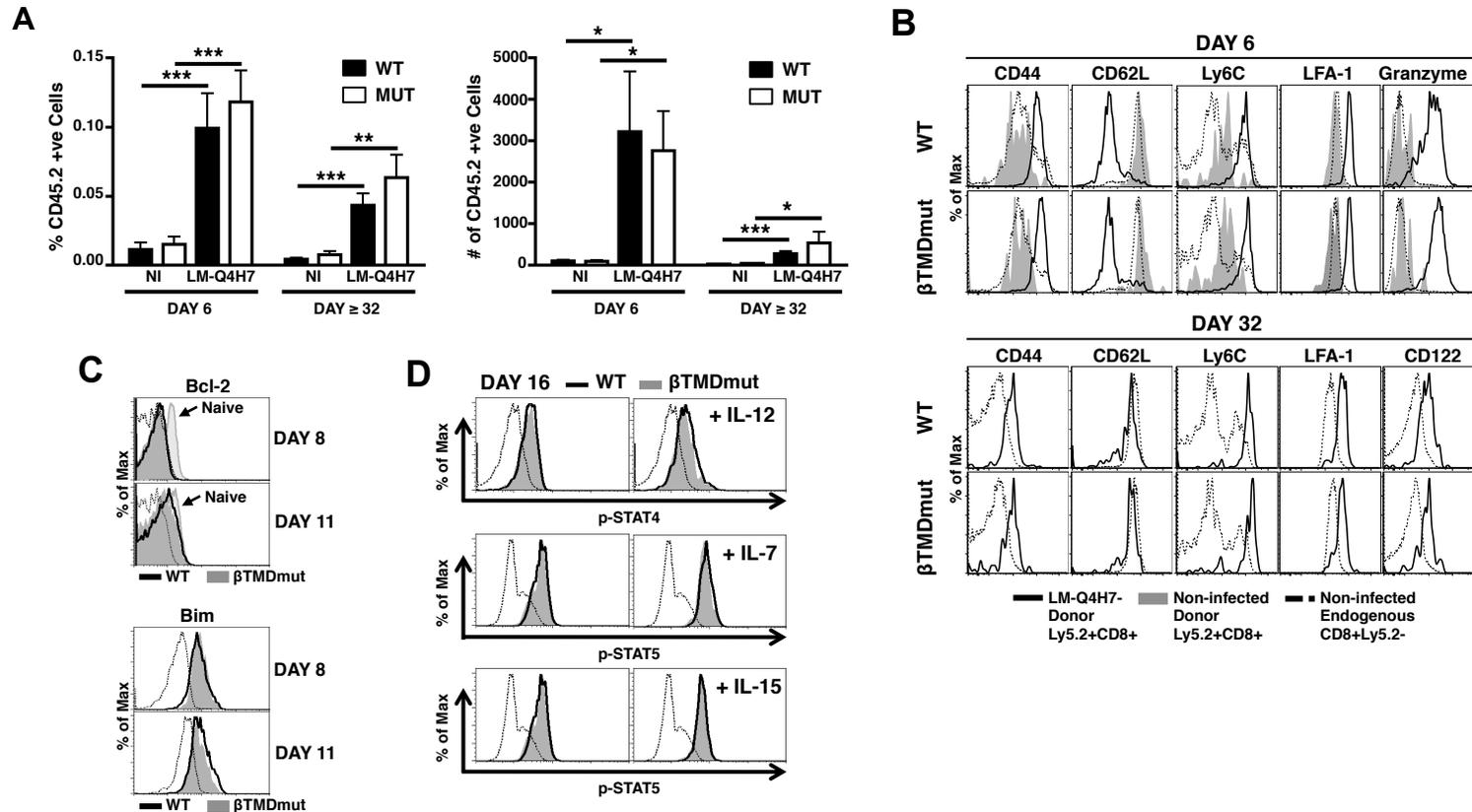


SUPPLEMENTAL FIGURE 1

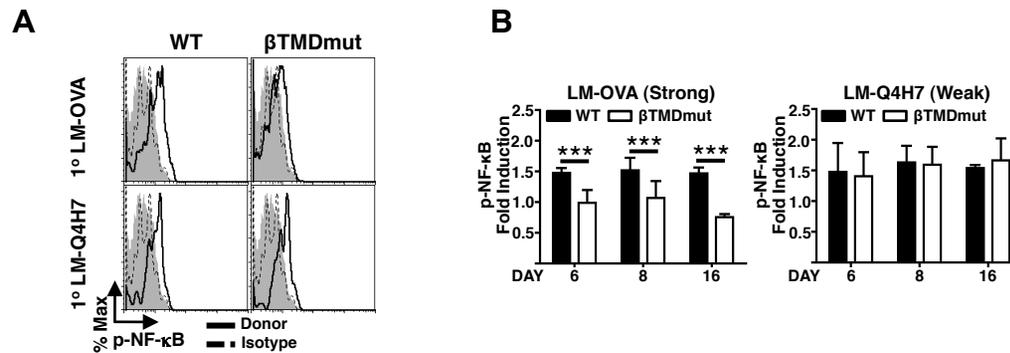


A and B. WT and MUT cells become activated and generate memory T cells in response to LM-Q4H7. 10^3 naïve WT or MUT donor CD45.2⁺CD8⁺ T cells were adoptively transferred into CD45.1⁺ hosts and challenged with 10^3 CFU LM-Q4H7 or PBS (noninfected-NI). At day 6 or day ≥ 32 , CD45.2⁺ donor cells were subjected to magnetic bead enrichment (see Materials and Methods). CD45.2⁺CD8⁺ donors frequency, numbers (A) and phenotype (B) from the bound fraction were determined by flow cytometry. Due to lack of detectable non-infected donor cells at memory, endogenous CD8⁺ T cells from non-infected mice were used as controls at day ≥ 32 . Values in graphs in (A) are shown as mean \pm SEM (*p < 0.05, **p < 0.005, ***p < 0.001). Graphs and histograms represent 3 independent experiments, n=3 mice each.

C. WT and MUT cells exhibit similar expression of anti- and pro-apoptotic factors in the contraction phase of the LM-OVA immune response. Mice were challenged as in Fig. 1. Bcl-2 and Bim expression of WT and MUT cells in the blood at day 8 and 11. Grey and dashed lines represent naïve and isotype control, respectively.

D. MUT cells are not defective in IL-12R, IL-7R or IL-15R signaling. Mice were challenged as in Fig. 1. Phosphorylation of STAT-4 and -5 determined by intracellular staining at day 16 of the LM-OVA response and upon stimulation with the cytokines are shown. Dashed line represents isotype control. Histograms representative of n ≥ 3 experiments, n = 3-5 mice each.

SUPPLEMENTAL FIGURE 2 VERSION 2



MUT cells are defective in the induction of NF- κ B signals in response to high affinity but not low affinity TCR ligands upon infection. A. WT or β TMDmut donor CD8 T cells were adoptively transferred into congenic B6 hosts and challenged with 1×10^3 CFU LM-OVA or LM-Q4H7. Histograms show phosphorylation of p65 NF- κ B (Ser 536) in antigen-specific T cells at the peak of the responses by flow cytometry. Solid grey histogram represents the naïve control. Histograms shown are representative of $n=4$ experiments, $n=3-6$ mice each. **B.** Graphs show induction of p-NF- κ B over naïve levels (mean \pm SD) at different days of the LM-OVA and LM-Q4H7 responses. Data is representative of $n \geq 3$ independent experiments; $n \geq 3$ mice per group. * $p \leq 0.05$, ** $p \leq 0.005$, *** $p \leq 0.001$.